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Binding Inhibition Between Igf-1r and Igf-1 by Catechin of Black Tea

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ABSTRACT

The natural compound of black tea is used as an alternative of obesity therapies in the world; particularly, the *catechin* family in tea leaves which has bioactive compounds such as EC, EGC and EGCG. Their bioactivity contributes to inhibit the ligand of Insulin-Like Growth Factor I Receptor (Igf-1r) binding-region to Igf-1 protein. To elucidate the inhibiton of *Igf-1* expression and proliferating of *Rattus norvegicus* strain wistar adipose cell using black tea solution. The research used *Rattus norvegicus* strain wistar. After a 90-day treatment, the adipose tissues were picked up from the viscera of each experimental animal, and then the adipose tissues were embedded by paraffin. The paraffin sections were determined through immunohistochemistry with anti-Igf-1 antiserum, and were also analyzed through hematoxylin-eosin. A protein sequence of Igf-1, Igf-1r, and 3D structure of EC, EGC and EGCG from Gene Bank sites were used during *in silico* analysis. The sequences were aligned by BLAST program to identify the conserve and variable domain of IGF-1 protein isoforms. The 3D structures of IGF-1 and IGF-1R were constructed using Phyre program. The ligand among the 3D structures of IGF-1, IGF-1R and catechin compounds were analyzed using Hex 5.1 docking program. The data showed that the Igf-1 expression of adipose cells was reduced at 0,03 g/ml BTS and 0,045 g/ml BTS treatments. The result of BLAST analysis showed that IGF-1 (a, b, c, and d) isoforms conserved a domain from amino acid no 22 until 134; and this region was a variable region. The EGCG bound L1 domain of IGF-1R with E-total -235.3 KJ/mol which was lower than EC (-208,4 KJ/mol) and EGC (-142 KJ/mol). The total energy of IGF-1 (a, b, c, but not d isoform) which interacted with EGCG was around -223.7 KJ/mol, EC is -205.6 KJ/mol and EGC was -191.7 KJ/mol. However, EC, EGC and EGCG was only able to prevent the interaction between the L1 of IGF-1R with IGF-1 protein, but not the opposite.

Keywords: *Adipose cell, black-tea, proliferation, catechin, IGF-1, IGF-1R*

INTRODUCTION

Fat distribution in our body is one of the causes of obesity [1]. Adipose cells secrete Insulin-like growth factor-I; it is originally described as a hepatic derived factor produced in response to growth hormone (GH), which then mediates the effects of GH on somatic growth (proliferation and differentiation [3]. Black tea (Camellia sinensis) solution has been used in weight loss treatment. It contains catechin which is a type of polyphenols. Some of those catechin are (-)epicatechin (EC), (-)Epigallocatechin (EGC) and (-)epigallocatechin-3-gallate (EGCG) [7].

This study aims to determine the inhibitation of Igf-1 by catechin *in vivo, in vitro* and *in silico* process. The data of this study can be used for further studies on bioactivity content in black tea solution as a natural agent therapy for obesity.

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MATERIALS AND METHODS

Black tea solution preparation

We used Natural Exclusive Taste Black tea (*Camellia sinensis)* which is produced by the Medical Herb Centre Co. There are three kinds of black tea solution (BTS) concentration: 0.015 g/ml, 0.03 g/ml, and 0.045 g/ml. All of the tea groups were boiled for about 15 minutes and were then purified with a tea filter. Filtrates were kept to be used for treatments.

Experiment Animal

Twelve male *Rattus norvegicus* strain wistar, 6 to 8 week old, about 200 gram in weight, were acclimated for a week in laboratory and fed with normal diets (contained 9.75 % of fats). Rats

were grouped in 4 different groups (each group contained of 3 rats), and were fed with high fat diet (contained 30.10% of fats) for *in vivo* experiments. Those groups are: Group A as the control group (high fat diet, no BTS), Group B (high fat diet, BTS 0.015 g/ml), Group C (high fat diet, BTS 0.030 g/ml) and Group D (high fat diet, BTS 0,045 g/ml). Those treatments were given every day for 90 days, and all rats were weighed everyday, too. After the treatments were finished, the rats were killed. To analyze Immunohistochemistry, adipose tissues were removed from viscera and were then kept on paraformalindehyde 4% storage at 4° C.

Immunohistochemistry.

To identify Igf-1 expression on adipose cells and proliferation of adipose cells, adipose tissues were processed to paraffin blocks. Paraffin blocks were cut to gain microscopic tissue slides. Immunohistochemistry staining was carried out following the protocol using anti-Igf-1 antiserum (1:800) for the first antibody and Biotin Goat Antimouse IgG (1:500) for thesecond antibody. The adipose cells were visualized by DAB under Stereo Microscope (Nikon) unit camera. Haematoxilin & Eosin staining were used to stain control preparats.

In silico analyzes

Amino acid Igf-1 ligand and Igf-1r were prepared by UniProt genebank and analyzed by Protein Homology/analog Y Recognition Engine (Phyre) version 2.0 to get the 3D structures. Catechin 3D structure for (-) Epicatechin (EC), (-) Epigallocatechin (EGC) and (-) Epigallocatechin-3-Gallate (EGCG) were obtained by National Centre of Biotechnology Information (NCBI). Alignment studies were performed with Basic Local Alignment Search Tools (BLAST) program from NCBI. The docking studies were performed with the Hex Protein Docking Version 5.0.

Statistics

One way ANOVA was employed first and then continued with Tukey-test to compare between black tea treatments effects to Igf-1 expression on adipose cells, adipose cells proliferation and rat weight. Statistical significance was accepted for $P<0.05$.

Ethics

The protocol for animal subjects in this research was approved by the Institutional Review Board of Brawijaya University (Malang, Indonesia) which is a branch of National Bioethics Commission (Indonesia).

RESULT AND DISCUSSION

Rats' weight

In the present study, rats were fed with high fat diet and weighed every day for 90 days to make them obese. Weight averages were 148,613 g for group A, 134,840 g group for B, 126,867 g for group C, and 110,087 g for group D. It showed that black tea solution (BTS) could decrease rat weight (Fig 1.).

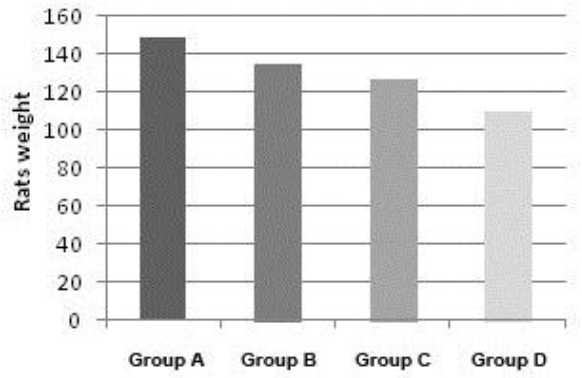

Fig.1. The avearage rat weight after 90 day treatment with black tea solution.

The highest BTS concentration gave the most effort to decrease rat weight compared to the control group. Previous study showed that adipose cell cultures have demonstrated inhibition of adipogenesis and increased apoptosis in treatment of EGCG 200 μM [6].

Proliferation and Igf-1 expression in adipose cells

We used Immunohistochemistry to evaluate the significance of BTS treatment effects on decreasing Igf-1 expression. Igf-1 expression was expressed by brown spots in membrane cells (Fig. 2. A, B, C & D). The most significant decreasing number of adipose cells that express Igf-1 are group with BTS treatment 0,03 g/ml and 0,045 g/ml. It has been reported that purified EGCG and TF-3 are potential inhibitors of epidermal growth factor receptor. These tea polyphenols were demonstrated to inhibit binding of cognate ligand to its receptor [4].

This study showed that BTS treatment could decrease proliferation of adipose cells, in which the most significance decrease were for groups with BTS treatment of 0,03 g/ml as many as 88 cells and 0,045 g/ml as many as 52 cells ($p<0,05$). These analyses showed that BTS inhibits Igf-1 ligand binding to its receptor in

adipose cells, causing tyrosine kinase to be not activated and causing signal transduction that stimulates adipose cells to everlasting proliferation.

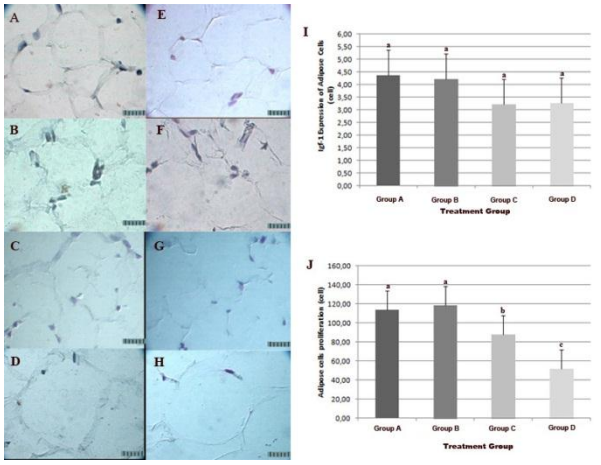

Fig.2. Adipose cells express Igf-1 by immunohistochemistry (A-D), H & E (E-H). *Bar Scale*: 5 µm, 400x. Amount of adipose cells that express Igf-1 (I), Amount of adipose cells proliferation (J).

Four sequences of Igf-1 proteins were gotten from Genebank National Centre Biotechnology Information (NCBI) [2] (Fig.3.)

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Isoform a preprotein: ---------- ------msap pikihimsss hlfylalcll tftssatagp
Isoform b preprotein: ---------- ------msap pikihimsss hlfylalcll tftssatagp
Isoform c preprotein: mgkisslptq lfkiclcdfl kikihimsss hlfylalcll tftssatagp
Isoform d preprotein: mgkisslptq lfkiclcdfl kikihimsss hlfylalcll tftssatagp

Isoform a preprotein: etlcgaelvd alqfvcgprg fyfnkptgyg ssirrapqtg ivdeccfrsc
Isoform b preprotein: etlcgaelvd alqfvcgprg fyfnkptgyg ssirrapqtg ivdeccfrsc
Isoform c preprotein: etlcgaelvd alqfvcgprg fyfnkptgyg ssirrapqtg ivdeccfrsc
Isoform d preprotein: etlcgaelvd alqfvcgprg fyfnkptgyg ssirrapqtg ivdeccfrsc

Isoform a preprotein: dlrrlemyca plkptksars iraqrhtdmp ktqksqplst hkkrklqrrr
Isoform b preprotein: dlrrlemyca plkptksars iraqrhtdmp ktqkevhlkn tsrgsagnkt
Isoform c preprotein: dlrrlemyca plkptksars iraqrhtdmp ktqksqplst hkkrklqrrr
Isoform d preprotein: dlrrlemyca plkptksars iraqrhtdmp ktqkevhlkn tsrgsagnkt

Isoform a preprotein: kgstleehk (ID: NP_001075946.2)
Isoform b preprotein: ymn------ (ID: NP_849197.3)
Isoform c preprotein: kgstleehk (ID: NP_001075947.1)
Isoform d preprotein: ymn------ (ID: NP_001075948.1)
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Fig.3.BLAST analysis: using four kinds of sequences of Igf-1 preprotein

Red amino acid (conserved area) showed the same amino acid sequence, and black amino acid (variable area) showed diferent amino acid sequences. Igf-1 isoform a preprotein and b preprotein has a same amino acid sequence in the order of 6 to 118, while Igf-1 isoform c preprotein and d preprotein has a same amino acid sequence in the order of 22 to 135. Isoform protein is a part of transduction signal in intracellular system. Those proteins have the same function with other proteins, but are encoded by different genes and probably have little difference with their sequences [8].

Docking Igf-1 with catechin and Igf-1r with catechin

3D structures for Igf-1 and Igf-1r were analyzed using Pyhre sites. Those analysis showed that 3D structure models of Igf-1 was a template structure from d1wqji1.model.pdb and Igf-1r is a template structure from c1igrA_.model.pdb. Based on the docking results, it was known that there were more than 1000 sequence solution which was docked with minimal binding energy of Igf-1r with Igf-1 at the top side (L1 domain) was -497.5 Kh/mol, RMS-1.00. It showed that Igf-1 was easily bound in L1 domain of Igf-1r because that kind of binding only needs a small energy resulted by some little parts of amino acid sequences (Fig4.). (-) epicatechin (EC), (-) Epigallocatechin (EGC) and (-) epigallocatechin-3-gallate (EGCG) were the mostly found bioactive molecule in tea, but EGCG amount was much more than EC and EGC amount. Based on NCBI [2], we got 3D structures of EC (Compound ID: 72276), EGC (Compound ID: 72277) and EGCG (Compound ID: 65064) (Fig.5.(I)).

No	Igf-1 r	Igf-1
1	Lys 169	Tyr 72
2	Pro 170	Phe 73
3	Met 171	Asn 74
4	Cys 172	Pro 76
5	Glu 173	Thr 77
6	Lys 174	Gly 78
7	Lys 193	Tyr 79
8	Met 194	Gly 80
9	Cys 195	Ser 81
10	Pro 196	Ser 82
11	Ser 197	Iie 83
12	Glu 206	Arg 84
13	Asn 207	Ala 86
14	Asn 208	Pro 87
15	Glu 209	Gln 88

Fig.4. Amino acids in Igf-1r binding Igf-1.

Based on the results from Igf-1r docking with EC, EGC and EGCG, there were 1000 sequence solution in bind with minimum E-total binding. Three kinds of catechin was known to bind easily in a same area; they were L1 domains even though every catechin needed different amount of energy (Fig.5.(II)). The amount of energy needed by EGCG to bind with Igf-1r was the smallest, (-235.3 Kj/mol, RMS -1.00), followed by the amount of energy needed by EC (-208,4 Kj/mol, RMS -1.00) and the amount of energy needed by EGC (-147 Kj/mol, RMS -1.00). Catechin could also bind with Igf-1. The smallest E-total binding energy was for EGCG (-223.7 Kj/mol, RMS -1.00), followed by EC (-205.6

Kj/mol, RMS -1.00) and EGC (-191.7 Kj/mol, RMS -1.00) (Fig.5.(III)).

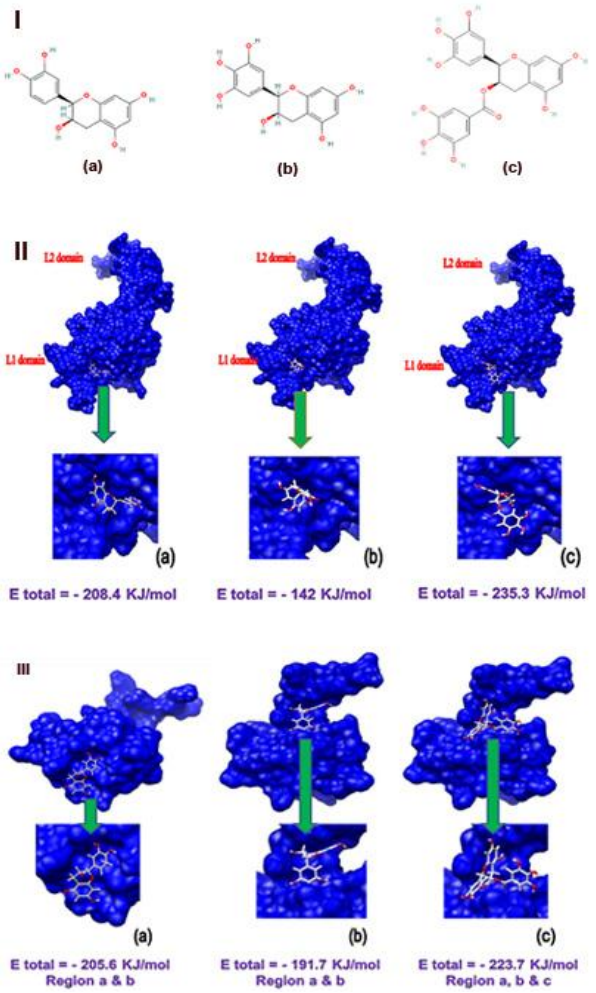

Fig.5.*Catechin* chemical structure (I), *Catechin* docking with Igf-1r (II), *Catechin* docking with Igf-1 (III).

Based on Chimera analysis, it was known that EGCG could bind isoform a, b and c of Igf-1, meanwhile EC and EGC could only bind isoform a and b. This analysis also showed that EGCG could bind Igf-1r and Igf-1 even though the energy required was still larger than the energy required by Igf-1 to bind Igf-1r. That three catechin could bind the active side of Igf-1r and the same side of Igf-1r where the Igf-1 bound Igf-1r. EGCG could be a competitor for ATP in binding Igf-1r, so it can inhibit transduction signal intracellular [5]

CONCLUSION

The result of this study showed that the catechin (EC, EGC and EGCG) inhibition was on IGF-1R but not IGF-1. It slightly inhibited the Igf-1 expression and proliferation of adipose cells. It showed that black tea has a high potential bioactivity as a natural therapy agent to prevent obesity.

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